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Adenosine receptors: therapeutic aspects for inflammatory and immune diseases

György Haskó[*], **Joel Linden**[‡], **Bruce Cronstein**[§], and **Pál Pacher**[‖]

[*]*Department of Surgery, University of Medicine and Dentistry of New Jersey-New Jersey Medical School, 185 South Orange Avenue, Newark, New Jersey 07103, USA.*

‡*Department of Medicine and the Cardiovascular Research Center, MR5 BOX 801394, University of Virginia, Charlottesville, Virginia 22908, USA.*

§*Department of Medicine, New York University School of Medicine, 550 First Avenue, New York, New York 10016, USA.*

‖*Section on Oxidative Stress Tissue Injury, Laboratory of Physiological Studies, National Institute on Alcohol Abuse and Alcoholism, 5625 Fishers Lane, MSC-9413, Bethesda, Maryland 20892-9413, USA.*

Abstract

Adenosine is a key endogenous molecule that regulates tissue function by activating four G-protein-coupled adenosine receptors: A_1, A_{2A}, A_{2B} and A_3. Cells of the immune system express these receptors and are responsive to the modulatory effects of adenosine in an inflammatory environment. Animal models of asthma, ischaemia, arthritis, sepsis, inflammatory bowel disease and wound healing have helped to elucidate the regulatory roles of the various adenosine receptors in dictating the development and progression of disease. This recent heightened awareness of the role of adenosine in the control of immune and inflammatory systems has generated excitement regarding the potential use of adenosine-receptor-based therapies in the treatment of infection, autoimmunity, ischaemia and degenerative diseases.

In 1929, Drury and Szent-Györgyi first reported the concept of adenosine acting as an extracellular signalling molecule[1]. The authors found that when simple extracts of heart muscle and other tissues were injected intravenously into the whole animal they produced decreased heart rate and increased coronary blood flow. The active constituent of the extracts turned out to be adenosine, and since the 1980s adenosine has been used to slow down the heart of patients suffering from excessively increased heart rate caused by supraventricular tachycardia[2]. In addition, adenosine has been used as a diagnostic agent, that is, a coronary vasodilator, to assess coronary artery function in conjunction with radionuclide myocardial perfusion imaging[3]. Following the original discovery of the effects of adenosine on cardiac function, adenosine has been found to have regulatory roles in virtually every organ system studied.

Correspondence to G.H. e-mail: haskoge@umdnj.edu.

Competing interests statement
The authors declare competing financial interests: see web version for details.

DATABASES
IUPHAR Receptor Database: http://www.iuphar-db.org A_1 | A_{2A} | A_{2B} | A_3

FURTHER INFORMATION
ClinicalTrials.gov: http://clinicaltrials.gov/ct2/show/NCT00430300
CV Therapeutics: http://www.cvt.com/a_pipeline.html
King Pharmaceuticals: http://www.kingpharm.com/kingpharm/AboutUs/fastFacts.asp

Adenosine accumulates in the extracellular space in response to metabolic stress and cell damage (BOX 1), and elevations in extracellular adenosine are found in conditions of ischaemia, hypoxia, inflammation and trauma[4,5]. The rapid release of adenosine in response to tissue-disturbing stimuli has a dual role in modulating homeostasis. First, extracellular adenosine represents a pre-eminent alarm molecule that reports tissue injury in an autocrine and paracrine manner to surrounding tissue. Second, extracellular adenosine generates a range of tissue responses that can be generally viewed as organ protective thereby mediating homeostasis. Adenosine elicits its physiological responses by binding to and activating one or more of the four transmembrane adenosine receptors, denoted A_1, A_{2A}, A_{2B} and A_3 (BOX 1). A prominent body of evidence supports the notion that the ability of adenosine, acting at its receptors, to control the immune and inflammatory systems plays a key role in the modulatory effects of adenosine in both health and disease. There are many promising emerging therapeutic approaches that are centred on the modulation of adenosine in the immune system. These encompass pharmacological compounds that interfere with the breakdown and generation of adenosine, as well as selective agonists and antagonists of various adenosine receptor subtypes (FIG. 1). Some of these compounds are in preclinical investigations, whereas others have already entered clinical trials for various indications. In this Review, we provide a general overview of adenosine receptors, covering aspects of cell biology, molecular biology and pharmacology. Separate sections focus on the multitude of roles that adenosine has in dictating the function of cell types that are considered basic constituents of the innate and adaptive immune systems. Finally, we discuss the therapeutic basis of harnessing the adenosine receptor system in managing patients suffering from inflammatory, autoimmune and ischaemic diseases.

Adenosine receptor signalling

A large body of evidence supports the view that adenosine receptors govern cell function by coupling to G proteins, although some G-protein-independent effects have also been reported[6]. Traditionally, adenosine receptor signalling is thought to occur through the inhibition or stimulation of adenylyl cyclase with a concomitant decrease or increase in intracellular cyclic AMP concentrations. Based on their ability to decrease or increase cAMP accumulation, adenosine receptors were initially classified as A_1 or A_2 receptors, respectively[7]. Subsequent studies have refined the classification of adenosine receptors and cAMP-increasing A_2 receptors have been divided into two groups: high-affinity A_{2A} receptors and low-affinity A_{2B} receptors[8]. The more recent discovery and characterization of A_3 receptors have made it clear that in addition to A_1 receptors, A_3 receptors dictate certain cellular responses such as rodent mast-cell degranulation, in part by decreasing intracellular cAMP concentrations[9]. This early picture of adenosine receptor signalling through the adenylyl cyclase–cAMP system has been substantially expanded, and it is now established that adenosine receptors can be linked to various other pathways.

Box 1

Adenosine-receptor metabolism and signalling

Extracellular adenosine levels increase following the release of adenosine from cells or as a result of extracellular catabolism of released adenine nucleotides. Intracellular adenosine, which can be derived from increased intracellular metabolism of ATP during cellular stress or *S*-adenosyl homocysteine, is liberated mainly via equilibrative nucleoside transporters. Extracellular ATP and ADP are catabolized by a cascade of ectoenzymes comprising CD39 (ENTPD1; ectonucleoside triphosphate diphosphohydrolase 1) and CD73 (ecto-5′-nucleotidase). CD39 is an enzyme that hydrolyses ATP and ADP to AMP, and CD73, in turn, rapidly dephosphorylates AMP to adenosine[126]. Owing to the ubiquitous presence of equilibrative nucleoside transporters, adenosine generated from extracellular ATP is rapidly

taken up by cells from the extracellular space. Adenosine in the cytosol is then metabolized either by adenosine kinase to form AMP or adenosine deaminase, which deaminates adenosine to inosine[6,127]. As a result of the rapid uptake and metabolism of adenosine, levels of this mediator are maintained low in unstressed, healthy tissues. However, under pathophysiological conditions adenosine removal can not keep pace with its generation, resulting in markedly increased extracellular adenosine concentrations.

All of the adenosine receptors contain seven transmembrane domains and couple to intracellular GTP-binding proteins (G proteins). Adenosine can activate A_1, A_{2A} and A_3 receptors with EC_{50} values that are between 0.01 μM and 1 μM, whereas A_{2B} receptor activation generally requires adenosine levels that exceed 10 μM (EC_{50} of 24 μM)[128]. Because physiological adenosine concentrations are lower than 1 μM, physiological levels of adenosine can activate A_1, A_{2A} and A_3 receptors, whereas A_{2B} receptor activation requires pathophysiological conditions[129]. Although the cellular responses to adenosine are highly dependent on the adenosine concentrations at the cell surface, several other factors, such as receptor density and the functionality of the intracellular signalling pathways coupled to adenosine receptors, are also key determinants in dictating the nature and magnitude of the effect of adenosine on the cell. For example, A_{2A} receptor activation potently suppresses the production of the T helper 1-inducing cytokine interleukin 12 (IL12) by monocytes that are preincubated with the pro-inflammatory cytokine IL1 or tumour-necrosis factor-α, agents that also upregulate A_{2A} receptors in these cells[119]. This example highlights the importance of receptor expression in defining the magnitude of the response to receptor stimulation. Furthermore, the effect of adenosine can also depend on the polarized localization of adenosine receptors. For example, A_3 adenosine receptors accumulate at the leading edge of migrating neutrophils and have an important role in facilitating the directional movement of cells in response to chemotactic stimuli[35]. Finally, caution should be exercised in interpreting data assessing adenosine receptor function in different species, because sequence variations in cloned adenosine receptors have been shown to be associated with varying pharmacological responses to selective agonists and antagonists. This is best illustrated by the fact that A_3 receptors remained undiscovered until they were cloned[130], because the rodent A_3 receptor is insensitive to xanthines such as theophylline and caffeine, antagonists that had been extensively used in identifying adenosine receptor-mediated effects.

A_1 receptor activation had traditionally been linked to G_i-mediated inhibition of adenylyl cyclase. However, it is now known to be also linked to various kinase pathways including protein kinase C (PKC), phosphoinositide 3 (PI3) kinase and mitogen-activated protein (MAP) kinases[10]. In addition, A_1 receptor activation can directly activate K^+ channels and inhibit Q-, p- and N-type Ca^{2+} channels. It is noteworthy that most of these signalling pathways were uncovered in non-immune cell types, and A_1 receptor signalling mechanisms in cells of the immune system are not known.

A_{2A} receptors, similar to other G_s-protein-coupled receptors, signal mainly by the adenylate cyclase–cAMP–protein kinase A (PKA) canonical pathway, but they can also signal through the activation of an exchange factor that is directly activated by cAMP (Epac)[6]. Signalling downstream from PKA proceeds through phosphorylation of the transcription factor CREB on serine residue 133, resulting in its activation[11]. Activated CREB can mediate gene expression directly by interacting with gene promoters or indirectly by competing with nuclear factor-κB (NF-κB) or other transcription factors for an important cofactor, CBP[6]. In other cell types A_{2A} receptors have been shown to signal for increased collagen production by activating MAP kinases[12] and inhibiting neutrophil superoxide production by activating protein phosphatase[13]. Furthermore, recent results indicate that CEBPβ is responsible for the stimulatory effect of A_{2A} receptor agonists on interleukin 10 (IL10) production by

macrophages[14] (FIG. 2). which of the outlined mechanisms mediate A_{2A} receptor signalling in the various immune cell types remains to be defined in more detail.

A_{2B} receptor stimulation can trigger adenylyl cyclase activation via G_s and phospholipase C (PLC) activation via G_q (REF. 15). Cross-talk between these two pathways appears to be essential for the upregulation of IL4 production by human mast cells following A_{2B} receptor activation[16]. In particular, G_q-mediated activation of PLCβ leads to calcium mobilization and an increase in NFATc1-dependent IL4 transcription, and this response is amplified by the G_s-mediated accumulation of NFATc1 protein.

The classical signalling pathways associated with A_3 receptor activation comprise G_i-mediated inhibition of adenylyl cyclase and G_q-mediated stimulation of PLC[17]. In addition, A_3 receptors can utilize the PLD, RhoA, WNT, MAP kinase and PI3 kinase pathways in dictating cell function. In fact, A_3 receptor-dependent enhancement of histamine release in sensitized murine mast cells was prevented by inhibition of G_i proteins using pertussis toxin and by pharmacological inhibition of PI3 kinase[18].

G_i

An α-subunit of a heterotrimeric GTP-binding and GTP-hydrolysing protein (G protein) that inhibits the activity of a downstream enzyme such as adenylyl cyclase.

G_s

An α-subunit of a heterotrimeric GTP-binding and GTP-hydrolysing protein (G protein) that stimulates the activity of a downstream enzyme such as adenylyl cyclase.

Adenosine receptors on immune cells

Macrophages

The effect of adenosine on cytokine production by macrophages has attracted considerable attention, because macrophage-derived cytokines are crucial initiators and orchestrators of immune responses. As tumour necrosis factor-α (TNF-α) was one of the first cytokines to be discovered, a substantial body of information has accumulated regarding the ability of adenosine receptor activation to limit TNF-α production following macrophage activation. Recent studies using adenosine-receptor knockout mice have painted a detailed but still not complete picture of the receptors involved. Several studies agree that the A_{2A} receptor is the primary and dominant adenosine receptor subtype that mediates inhibition of TNF-α[19-21]. A role for other receptors was postulated based on the observation that adenosine, and the agonists NECA and IB-MECA (CF-101) can each inhibit, albeit to a lesser extent, TNF-α production even in A_{2A}-receptor knockout mice[19,20]. A study using a combined approach of using A_{2A}-receptor knockout mice and the A_{2B} receptor antagonist MRS 1754 supports a role for A_{2B} receptors as the other inhibitory receptor[20]. However, it appears that A_{2B} receptors become operational only when their effect is not masked by A_{2A} receptors, because both MRS 1754 and genetic deletion of A_{2B} receptors in the presence of functional A_{2A} receptors fails to affect the suppression of TNF-α production[20,21].

G_q

An α-subunit of a heterotrimeric GTP-binding and GTP-hydrolysing protein (G protein) that stimulates the activity of a downstream enzyme such as phospholipase C.

A similar picture is emerging regarding how adenosine receptor activation augments IL10 production. A key role for A_{2A} receptors was recently documented when adenosine failed to upregulate *Escherchia coli*-induced IL10 release by macrophages lacking A_{2A} receptors but not wild-type macrophages[14]. In the RAW264.7 macrophage cell line, which expresses negligible levels of A_{2A} receptors, adenosine upregulates IL10 production via an A_{2B}-receptor-mediated mechanism[22]. while A_{2B} receptor activation has a minor impact on TNF-α and IL10 production, it is key to the stimulatory effect of adenosine on IL6 production, as NECA is unable to induce the release of IL6 in A_{2B}-receptor knockout but not wild-type macrophages[21].

Finally, it is important to emphasize that the issue of which adenosine receptors regulate cytokine production by human monocytes/macrophages is even more contentious with evidence pointing to the involvement of all four receptors[23]. These results, however, must be interpreted with caution, because they are based entirely on pharmacological approaches, and several of the ligands used, especially in early studies, are not particularly selective. Nevertheless, the recent observations that human newborn plasma contains elevated concentrations of adenosine when compared with adult plasma, and degrading adenosine with adenosine deaminase or interrupting adenosine signalling by adenosine receptor blockade augments TNF-α production by neonatal but not adult blood, highlight the importance of the inhibitory effect of adenosine on TNF-α production in human immunity[24]. Based on these results it was speculated that adenosine might protect the fetus from excessive inflammatory responses that drive alloimmune reactions and premature delivery. However, this protective effect would come at a price of increased susceptibility to infections following birth.

Dendritic cells

Although dendritic cell responses from adenosine-receptor knockout mice have yet to be characterized, available data from human studies support a consistent role for adenosine receptors in orchestrating dendritic cell function. G_i-coupled (A_1, A_3 or both depending on the experimental system) adenosine receptors are expressed on both immature myeloid[25] and plasmocytoid dendritic cells[26], and their activation results in the mobilization of intracellular calcium from intracellular stores and reorganization of the actin cytoskeleton. Consistent with these adenosine-induced intracellular responses, immature dendritic cells migrate along different concentration gradients of adenosine, indicating that adenosine receptor activation induces chemotaxis in immature dendritic cells[25,26]. These stimulatory responses induced by G_i-coupled adenosine receptors can be observed only in immature dendritic cells, as these receptors undergo downregulation during dendritic cell maturation[25,26].

Although A_{2A} receptors are present on immature dendritic cells, they are expressed at low levels and appear to be silent, as their activation is unable to elicit downstream signalling events such as accumulation of intracellular cAMP[25]. However, dendritic cell maturation is accompanied by the emergence of A_{2A}-receptor-mediated signalling responses, owing to both increased expression and coupling of A_{2A} receptors[25,26]. A_{2A} receptor activation on mature dendritic cells shifts their cytokine profile from a pro-inflammatory to an anti-inflammatory one, with reduced IL12, IL6 and interferon-α (IFN-α) production and augmented IL10 production[25-27]. It is likely that dendritic cells in the presence of adenosine have a reduced capacity to induce T helper 1 (T_H1) cell versus T_H2 cell polarization of naive $CD4^+$ cells[27]. This is due to the adenosine-induced switch in dendritic cell cytokine production away from the T_H1-inducing IL12 towards the T_H2-inducing IL10.

In summary, the available data support a dual role for adenosine in dictating dendritic cell function. Adenosine promotes the recruitment of immature dendritic cells to sites of inflammation and injury via A_1 or A_3 receptors. At these sites adenosine produces, via A_{2A} receptors, an anti-inflammatory dendritic cell phenotype driving T-cell responses towards a T_H2 profile.

Neutrophils

Adenosine is a potent modulator of neutrophil function and it has long been appreciated that adenosine, by activating its receptors, regulates stimulated production of reactive oxygen species by these cells and phagocytosis[28-30].

Individual neutrophils do not produce large quantities of cytokines; however, because of the large numbers of accumulated neutrophils the cumulative contribution to pro-inflammatory cytokine levels at a given site is large. Adenosine, acting at A_{2A} receptors, regulates the production of a range of cytokines including TNF-α, macrophage inflammatory protein (MIP)-1α (also known as CCL3), MIP-1β (CCL4), MIP-2α (CXCL2) and MIP-3α (CCL20) [31]. Neutrophils are recruited to inflammatory sites by the post-capillary venular endothelium, which alters the expression of adhesive molecules on its surface to capture neutrophils from the circulation. Adenosine, via A_{2A} receptors, inhibits the adhesion of neutrophils to the endothelium by decreasing the expressionand stickiness of the adhesion molecules expressed on neutrophils[32-34].

NFATc1

NFATc1 is a member of the nuclear factor of activated T cells (NFAT) protein family, which are a family of transcription factors whose activation is controlled by calcineurin, a Ca^{2+}-dependent phosphatase. They were originally identified in T cells as inducers of cytokine gene expression.

Pertussis toxin

A compound that inhibits the guanine nucleotide binding proteins G_i and G_o via ADP ribosylation.

Macrophage

One of the main types of professional phagocytes. Macrophages are long-lived and detrimental for many microbial pathogens. Intracellular bacteria can survive within the macrophages. They can mediate antibody-dependent cellular cytotoxicity through phagocytosis.

By contrast, A_1 receptors promote neutrophil adhesion to different adhesive molecules on the endothelium and on other surfaces[32]. Once in the tissue neutrophils migrate along gradients of chemoattractants. There are many chemoattractants including activated complement components (C5a), bacterial products (formylated peptides) and chemokines, and studies have shown that adenosine promotes directed migration of neutrophils via A_1 and A_3 receptors[35-37]. In addition, neutrophils cluster their A_3 receptors at the leading edge of the cell and release ATP, which is converted at the cell surface to adenosine, which then acts in an autocrine manner to stimulate migration[35]. At inflamed sites neutrophils undergo apoptosis and adenosine, acting at A_{2A} receptors, prevents neutrophils from undergoing apoptosis[38-40]. Thus, virtually every function carried out by neutrophils is regulated by adenosine and its receptors.

Mast cells

The observation that inhaled adenosine provokes bronchoconstriction in individuals suffering from asthma but not in normal volunteers has propelled adenosine and adenosine receptors into the forefront of asthma research[41]. Although the mechanisms of how adenosine mediates bronchoconstriction are still elusive, there is overwhelming evidence that mast-cell mediators such as cytokines and histamine may have a key role in evoking airway constriction in response to adenosine. Although A_{2A}, A_{2B} and A_3 receptors are clearly present on the cell membrane of mast cells[42], there is uncertainty regarding which receptor(s) account for the increased mast-cell activation following adenosine receptor activation.

Adenosine was recently documented to stimulate release of the major asthma-promoting cytokine IL13 by mouse mast cells that were obtained from wild-type but not A_{2B}-receptor-knockout mice. This result implies that A_{2B} receptors are the receptors involved in the pro-inflammatory effects of adenosine[43]. In addition, it is clear from genetic studies with knockout mice that A_3 but not A_{2B} receptor agonism can produce histamine release of lung mast cells in the absence of specific antigenic stimulation[18]. Thus, A_{2B} and A_3 receptors subserve different pro-inflammatory roles in naive mouse mast cells. The role of A_{2B} receptors in regulating antigen-stimulated histamine release in mouse mast cells is controversial. Mast cells isolated from wild-type animals display decreased histamine release following stimulation with antigen when compared with mast cells obtained from A_{2B}-receptor knockout mice[44]. This observation was interpreted as indicating that constitutive activation of A_{2B} receptors on cultured mast cells suppresses the release of histamine. A subsequent study confirmed the increased degranulation of A_{2B}-deficient mast cells, but based on additional approaches the authors questioned this interpretation of A_{2B}-receptor-mediated decrease of degranulation of mast cells[43]. The reason for the different interpretation in the latter study was that exogenous adenosine maintained its stimulatory effect on histamine release in A_{2B}-receptor knockout mast cells[43]. In addition, no evidence of constitutive activity of A_{2B} receptors was revealed by studies using inverse agonists and measuring intracellular cAMP levels. Thus, it appears likely that the decreased release of histamine by mast cells isolated from A_{2B} receptor wild-type versus knockout mice reflects an altered responsiveness of A_{2B} receptor wild-type versus knockout cells to antigen. This is possibly from altered development of mast cells in A_{2B}-receptor-knockout mice. Unlike A_{2B} receptors, A_3 receptors directly control histamine release by antigen-stimulated mouse mast cells, because the stimulatory effect of exogenous adenosine noted in wild-type mast cells is not observed in A_3-receptor-knockout mast cells[45].

In canine and human mast cells, degranulation or cytokine release appears to be mediated primarily by A_{2B} receptor activation[46,47]. A_{2B} receptor stimulation in human mastocytoma HMC-1 cells induces secretion of the T_H2 cytokines IL4 and IL13, as well as a number of other pro-inflammatory cytokines such as IL1β and IL8. Although it is not clear whether adenosine receptor activation is able to stimulate the degranulation of human mast cells[44], the observation of increased IL4 and IL13 production following adenosine receptor activation strongly implicates human mast-cell adenosine receptors as important players in the pathophysiology of human asthma.

Lymphocytes

In addition to regulating lymphocyte function indirectly by stimulating adenosine receptors on innate immune cells such as dendritic cells, adenosine can also directly affect lymphocyte responses by binding and activating adenosine receptors on lymphocytes. A number of recent studies using adenosine-receptor-knockout mice have evaluated the effect of adenosine receptor activation on various lymphocyte functions. The consensus emerging from these studies, as well as pharmacological studies, is that A_{2A} receptors are the dominant adenosine receptors in dictating lymphocyte responses.

Studies using A_{2A}-knockout models have shown that A_{2A} receptor activation inhibits IL2 secretion[48] by naive $CD4^+$ T cells thereby reducing their proliferation[49] following T-cell receptor stimulation. A_{2A} receptor activation also suppresses the production of both IFN-γ and IL4 by both naive $CD4^+$ T cells[48,50] and polarized T_H1 and T_H2 cells[51], thus challenging the hypothesis that A_{2A} receptors on lymphocytes might shift T_H cell responses towards a T_H2 profile. Further immunosuppressive effects of A_{2A} receptor stimulation include the upregulation of the expression of negative co-stimulatory molecules such as cytotoxic T-lymphocyte antigen 4 (CTLA4) and programmed cell death 1 (PD1), and downregulation of the positive co-stimulatory molecule CD-40L[49].

Similar to $CD4^+$ cells, adenosine inhibits IL2 production by both polarized type 1 cytotoxic T (TC1) and TC2 $CD8^+$ cells, the effect of which was proposed to proceed through A_{2A} receptors based on pharmacological evidence[52]. However, the production of neither TC1 (IFN-γ) nor TC2 (IL4 and IL5) cytokines was influenced by A_{2A} receptor activation. In addition, pharmacological A_{2A} receptor activation failed to reduce TC1 or TC2 cell cytolytic function[52], which suggests that another subtype, possibly the A_3 receptor, may mediate the anti-cytotoxic effect of adenosine noted in prior studies[53,54]. In contrast to these results with $CD8^+$ cytotoxic cells, recent data with A_1-, A_{2A}- and A_3-receptor-knockout mice support a primary role for A_{2A} receptors in preventing the cytolytic activity of IL2-activated natural killer (NK) cells[55].

Adenosine deaminase

Adenosine deaminase irreversibly deaminates adenosine, converting it to the related nucleoside inosine by the removal of an amino group.

Alloimmune reaction

Alloimmunity is an immune reaction against non-self material from the same species.

Dendritic cell

These professional antigen-presenting cells are increasingly recognized as having crucial immunoregulatory functions. They are found in various tissues where they take up antigens, process them, migrate to the lymph nodes and present the antigens to T cells.

$CD4^+$ cells

Cells expressing the $CD4^+$ glycoprotein that recognises major histocompatibility class II molecules.

T_H1 and T_H2 cells

The T_H1/T_H2 hypothesis came to prominence in the late 1980s, indicating that mouse T-helper (T_H) cells broadly express differing cytokine profiles. T helper 1 (T_H1) cells secrete interferon-γ and tumour necrosis factor-α. T_H2 cells secrete interleukin 4 (IL4), IL5 and IL13. In addition, T_H3 and regulatory $CD4^+$ $CD25^+$ T cells exist that produce transforming growth factor-α and IL10, respectively.

Mast cell

A bone marrow-derived cell that is present in various tissues; they are important contributors to allergic disease and possibly arthritis. They are granular cells that bear Fc receptors for immunoglobulin E (IgE), which, when crosslinked by IgE and antigen, causes degranulation and release of mediators such as histamine, leukotrienes and PGD2.

Antigenic stimulation

When the body's immune system responds to a foreign substance.

Recent studies have revealed an important role for adenosine in mediating the immune suppressive properties of regulatory T (T_{Reg}) cells, a cell type that is thought to have a key role in keeping the immune system at bay, thereby preventing excessive tissue injury (FIG. 3). It was found that T_{Reg} cells — as defined by their expression of $CD4^+/CD25^+/FoXp3^+$ and an ability to suppress the proliferation of $CD4^+/CD25^-$ cells — express high levels of both CD39 (REF. 56) and CD73 (REFs 56,[57]), cell surface ectoenzymes that convert extracellular ATP and ADP to adenosine (BOX 1, FIG. 3). The finding that T_{Reg} cells selectively co-express CD39 and CD73 prompted the authors to propose that CD39 and CD73 could represent new specific markers of T_{Reg} cells. The mechanistic link between the transcription factor $FOXP3^+$ and CD39 is suggested by recent studies showing that $FOXP3^+$ actually drives the expression of CD39 (REF. 58).

The mechanisms of the immune suppressive effects of T_{Reg} cells have been elusive. In this regard, it was recently discovered that T_{Reg} cells can efficiently hydrolyse exogenous ADP to generate immune suppressive adenosine via CD39 and CD73 (REF. 56). Furthermore, the regulatory function of T_{Reg} cells depended largely on their ability to produce adenosine, because T_{Reg} cells isolated from CD39-knockout mice lost their potential to suppress proliferation of $CD4^+/CD25^-$ cells, the effect of which was reconstituted by addition of soluble exogenous forms of CD39 (REF. 56). Further support to highlight an important role for adenosine was provided by the observation that A_{2A}-receptor-knockout target cells ($CD4^+/CD25^-$) proliferated more than wild-type cells when cultured with wild-type T_{Reg} cells. It is noteworthy that $CD4^+/CD25^-$ cells increased their expression of A_{2A} receptor by day 4 in culture, at a time when T_{Reg}-cell-mediated suppression of target cell proliferation is highest, again confirming that adenosine generation is an important component of the T_{Reg}-cell armamentum. In addition to target cells, T_{Reg} cells also express A_{2A} receptors and the activation of these receptors upregulates $FOXP3^+$ expression in T_{Reg} cells[59]. The functional significance of A_{2A} receptors on T_{Reg} cells was demonstrated in a mouse model of colitis[48]. In this model, adoptively transferred T_{Reg} cells lacking A_{2A} receptors were found to be defective in their ability to suppress colitis. Hence, in an adenine-nucleotide-rich environment, T_{Reg} cells generate adenosine via ectoenzymes and are responsive to adenosine via A_{2A} receptors.

Conventional T cells are activated via major histocompatability complex (MHC) molecules on antigen-presenting cells that present antigens to T-cell receptors. The subsequent expansion of activated T cells is responsible for adaptive immunity. A subset of T cells known as invariant NKT (iNKT) cells plays an important role in the innate immune response that triggers rapid host responses to infection. These cells express an invariant α,β T-cell receptor (vα14Jα18 in mice) in addition to molecular markers found on NK cells, such as NK1.1 (REF. 60). Unlike conventional T cells that recognize peptides, iNKT cells recognize lipid antigens that are derived from pathogens or injured host tissue. These lipid antigens are presented by antigen presenting cells to iNKT cells by the MHC class I-related molecule CD1d[61]. A_{2A} receptors are found on iNKT cells and strongly suppress the release of pro-inflammatory cytokines such as INF-γ[62]. The presence of A_{2A} receptors on iNKT cells suggests that there could be novel therapeutic applications of A_{2A} agonists or antagonists as activation of iNKT cells has been implicated in a number of disease processes including atherosclerosis, type 1 diabetes, arthritis and various allergic diseases[63].

In summary, lymphocyte function is potently regulated by A_{2A} receptors, suggesting that the anti-inflammatory effects of A_{2A} receptor agonists in animal models of autoimmunity and ischaemia are mediated, in part, by targeting lymphocytes.

Overall effect of adenosine in inflammation

Asthma and COPD

Several lines of clinical and preclinical evidence support the notion that adenosine and its receptors are intimately involved in defining the pathophysiology of asthma and chronic obstructive pulmonary disorder (COPD). Adenosine receptors on immune cells contained in the lung appear to have particularly important roles. Most compelling are the observations that inhaled adenosine can induce bronchoconstriction in patients suffering from asthma or COPD, but not in healthy individuals, and adenosine receptor blockade can prevent this bronchoconstrictive response[42]. These observations, combined with the demonstration that both adenosine levels and adenosine receptor expression on immune cells in the lung are elevated in patients with asthma and COPD, implicate endogenous adenosine as a prominent signalling molecule in lung diseases. As the bronchoconstrictive response to adenosine can be blocked by mast-cell membrane-stabilizing agents that can prevent the release of injurious mast-cell mediators, and because histamine receptor antagonists are also effective against adenosine, one major hypothesis explaining the mechanism of adenosine-mediated bronchoconstriction centres around a key role played by mast cells[42]. This general scheme of the injurious role of adenosine receptor activation on mast cells has been reinforced by studies using rodent models of asthma. These studies have also identified A_{2B} (REF. 43) and A_3 (REFS 18,[45]) receptors as the main contributors that mediate the stimulatory effect of adenosine on mast-cell activation. Although there is a paucity of human *in vivo* studies detailing the role of adenosine receptor subtypes in asthma and COPD, *in vitro* studies using human mast cells have narrowed down the candidate adenosine receptors to the A_{2B} receptor (FIG. 4). In addition to mast cells, pro-inflammatory effects of A_{2B} stimulation have also been observed with human bronchial smooth-muscle cells[64], human bronchial epithelial cells[65] and human lung fibroblasts[66], which respond to adenosine by increased release of Il6 (REFS 64,[66]) and IL19 (REF. 65). A_{2B} receptor activation on human lung fibroblasts promotes their differentiation into myofibroblasts that are capable of overproducing extracellular matrix[66], which suggests that adenosine may participate in the fibrosis and remodelling of the lung during asthma and COPD.

Inverse agonists

Inverse agonists reverse constitutive receptor activity, and are proposed to show selectively higher affinity for the inactive versus the active conformation of the receptor. In the absence of constitutive activity, inverse agonists function as competitive antagonists.

Lymphocyte

White blood cells of lymphoid origin that function as part of the immune system.

TC1 and TC2 $CD8^+$ cells

$CD8^+$ T cells have been subdivided into $CD8^+$ T cells secreting a T_H1-like cytokine pattern, which are defined as TC1 (T cytotoxic type 1) cells, versus $CD8^+$ T cells secreting a T_H2-like pattern (TC2 cells).

This substantial evidence documenting the pro-inflammatory effects of selective A_{2B} receptor activation in human and rodent cellular systems combined with the utility of A_{2B} receptor antagonism in preventing disease progression in rodent animal models[67,68] suggest that A_{2B} antagonists may be a viable treatment option for the management of asthma and COPD in human patients. Indeed, it is now well established that enprofylline, an anti-asthmatic agent, is a relatively selective (albeit not potent) A_{2B} receptor antagonist[69]. The selective A_{2B}

receptor antagonist CVT-6883 (REFS 67,68), which has shown efficacy in preventing disease in rodent models of asthma and COPD, appeared to be safe and well tolerated in a recent Phase I study, raising hopes that this agent might have utility in the treatment of individuals suffering from asthma and COPD (TABLE 1).

Lung inflammation results in the accumulation of neutrophils and other leukocytes in the interstitial space and in the airway. This is accompanied by an increased microvascular permeability and the release of chemotactic cytokines. A_{2A} agonists have been found to reverse these effects. To determine which pulmonary cells respond to A_{2A} receptor activation, pulmonary inflammation has been assessed in wild-type and A_{2A}-receptor-knockout mice[70]. To differentiate the role of A_{2A} receptors on haematopoietic and parenchymal cells, chimeric mice were created by transfer of bone marrow between wild-type and A_{2A} receptor knockout mice. In wild-type mice, A_{2A} receptor activation reduces lipopolysaccharide-induced neutrophil recruitment and release of cytokines. Pretreatment, but not post-treatment, also reduces the increase in vascular permeability. A_{2A} receptor activation only reduced lung inflammation when the A_{2A} receptor was present on bone-marrow-derived cells. In addition, using A_{2A}-receptor-knockout mice, the adenosine–A_{2A} receptor–cAMP axis was identified as a potent endogenous anti-inflammatory signalling pathway that reduces airway reactivity and inflammatory-cell infiltration following sensitization with ragweed[71]. Thus, A_{2A} agonists appear to be effective at curbing inflammatory lung tissue damage. However, in clinical trials, the utility of the A_{2A} agonist GW328267X was limited by cardiovascular side effects[72] (TABLE 1).

Ischaemia

During reperfusion following ischaemic injury, many tissues have been shown to be protected from reperfusion injury owing to the activation of A_{2A} receptors on bone-marrow-derived cells (FIG. 5). These include liver[73], kidney[74], heart[75], skin[76], spinal cord[77,78] and lung[79]. In liver and kidney tissues iNKT cells have been found to be sensitive to A_{2A} receptor activation and to have a crucial role in initiating an inflammatory cascade during reperfusion injury. These cells are rapidly stimulated to produce IFN-γ within 2 hours after the initiation of reperfusion, and the use of antibodies to deplete NK1.1-positive cells (NK and iNKT) or to block CD1d-mediated lipid-antigen presentation to iNKT cells replicates, but is not additive to the protection from reperfusion injury afforded by A_{2A} adenosine receptor activation[62,80]. Liver reperfusion injury is also reduced in *Rag1*-knockout mice that lack mature lymphocytes, and can be restored to the level seen in wild-type mice by adoptive transfer of iNKT cells purified from wild-type or A_{2A} receptor knockout mice, but not IFN-γ-knockout mice[62]. Additionally, animals with transferred A_{2A}-knockout iNKT cells are not protected from hepatic reperfusion injury by A_{2A} receptor activation. *In vitro,* A_{2A} receptor activation potently inhibits activation of iNKT cells. These findings suggest that reperfusion injury is initiated by CD1d-dependent activation of iNKT cells, and the activation of these cells is inhibited by A_{2A} receptor activation. The activation of iNKT cells results in the recruitment and transactivation of other immune cells such as macrophages and neutrophils that propagate tissue inflammation and injury.

Natural killer (NK) cells

A lymphocyte subset that is part of the innate immune response and is able to recognize virus-infected or transformed cells that lack major histocompatibility class I expression. In contrast to T cells, NK cells do not require activation but are able to immediately kill these cells.

Regulatory T (T_{Reg}) cells

T cells are a $CD4^+$ T-cell subset that are characterized by the expression of CD25 (interleukin 2 receptor-(IL2R) subunit) and FOXP3. T_{Reg} cells are powerful suppressors of adaptive immune responses.

MHC molecules

Originally named because they function as transplantation antigens. MHC molecules have a crucial role in antigen presentation, and serve as accessory binding proteins for T-helper and T-killer cells.

Arthritis

Despite the introduction of a number of effective biological agents for the treatment of rheumatoid arthritis over the past decade methotrexate remains one of the most effective and most commonly used therapies for inflammatory arthritis. Although originally introduced for the treatment of cancer as a folic acid analogue, the mechanism by which methotrexate, at very low dosages (average dosage in the United States, 17.5 mg per week for rheumatoid arthritis versus as much as 5 g per week for cancer), diminishes inflammation differs from the anti-proliferative mechanism of the drug. At low doses methotrexate is taken up by cells and polyglutamated to a long-lasting metabolite[81]. Methotrexate Polyglutamates have a different spectrum of enzyme inhibition and AICAR transformylase, an enzyme that is part of the *de novo* purine synthetic pathway, appears to be most sensitive to inhibition by methotrexate polyglutamates[82]. At low doses of methotrexate, this leads to the intracellular accumulation of AICAR[83,84]. AICAR is a competitive inhibitor of AMP deaminase leading, ultimately, to enhanced adenosine release from cells[83]. Although it is difficult to measure adenosine levels in biological fluids owing to the short half-life (2–8 seconds) of adenosine in blood and other fluids[85] in patients with rheumatoid arthritis treated with methotrexate there is strong evidence that methotrexate therapy promotes adenosine release[86,87]. Studies in mice and rats demonstrate that the anti-inflammatory effects of methotrexate are mediated by adenosine and that the anti-inflammatory effect of methotrexate is lost if animals are treated with adenosine receptor antagonists or if their adenosine A_{2A} or A_3 receptors have been deleted[88-94]. Similar loss of methotrexate efficacy has been noted in patients with rheumatoid arthritis who ingest significant quantities of caffeine, an adenosine receptor antagonist[95]. Thus, by promoting adenosine release at inflamed sites methotrexate diminishes inflammation in patients with rheumatoid arthritis. Building on the observation that methotrexate exerts some of its beneficial effects via A_3 receptors, coupled with the finding that the selective A_3 receptor agonist IB-MECA ameliorates the course of arthritis in mouse models[96-98], IB-MECA was recently tested in a phase II trial in patients with rheumatoid arthritis. IB-MECA was safe and well tolerated, and patients treated with this drug achieved a moderate reduction of symptoms[99] (TABLE 1). Methotrexate and IB-MECA have additive anti-inflammatory effects in rat adjuvant arthritis, which are mediated by an upregulation of the expression of A_3 receptors on inflammatory cells[100].

Sepsis

Preclinical studies using both knockout and pharmacological approaches have provided insights into the role of the various adenosine receptors in the physiological response of the organism to sepsis[101]. Inactivation of A_1 receptors increased mortality in intra-abdominal sepsis provoked by cecal ligation and puncture in mice, an effect that was correlated with heightened inflammation-induced hepatic and renal injury[102]. These results suggest that A_1 receptor activation may have a beneficial effect and additional studies testing the efficacy of A_1 receptor agonists are warranted. Similar to results with A_1 receptor blockade in cecal ligation and puncture, both knockout and pharmacological antagonism of A_3 receptors increased mortality, whereas stimulation of A_3 receptors with IB-MECA was protective[103].

In contrast to observations with blockade of A_1 and A_3 receptors, A_{2A} receptor inactivation by either gene deletion or administration of ZM241385 prevented cecal ligation and puncture-induced mortality by a mechanism that involved decreased bacterial dissemination that appeared to be secondary to sustained immune system function[104]. Paradoxically, A_{2A} receptor activation, when combined with antibiotics, has been noted to reduce mortality from sepsis induced by injecting with live *E. coli*, possibly by suppressing an exaggerated inflammatory response that can result from the rapid drug-induced killing of large numbers of bacteria[105]. Recent preliminary studies evaluating the role of A_{2B} receptors have demonstrated an anti-inflammatory and protective role of A_{2B} receptor stimulation in cecal ligation and puncture (C. Csoka, G. Hasko, Z. H. Nemeth and p. Pacher, unpublished observations).

Invariant NKT (iNKT) cells

A rare subset of lymphocytes that expresses an invariant T-cell receptor that recognizes certain glycolipids when bound to the major histocompatibility complex class I-like molecule, CD1d. Through secretion of cytokines they are powerful modulators of adaptive immune responses.

Cecal ligation and puncture

An experimental model of polymicrobial sepsis that is generally considered more relevant to the human disease than rodents injected with bacterial lipopolysaccharide (endotoxin).

Inflammatory bowel disease

Crohn's disease and ulcerative colitis are chronic, relapsing inflammatory bowel diseases (IBDs) that are characterized by a dysfunction of mucosal T cells, imbalanced cytokine production and cellular inflammation leading to damage of the intestinal mucosa. Although the aetiology of IBD remains to be determined, recent studies suggest that disease results from an inappropriately regulated immune response to luminal antigens in a genetically susceptible host[106]. $CD4^+CD25^+$ T_{Reg} cells have emerged as master regulators of the inflammatory response of the mucosa in IBD and have also been documented to offer a therapeutic option in severe inflammatory colitis[107]. Studies have highlighted the protective effects of A_{2A} receptor activation in various animal models of colitis, and these protective effects can be ascribed to two major mechanisms: decrease of inflammatory-cell infiltration and function in the mucosa, and increased activity of T_{Reg} cells. The first mechanism is supported by evidence that A_{2A} receptor activation by ATL146e (an A_{2A} receptor agonist) decreases both leukocyte infiltration and the production of inflammatory cytokines by disease-inducing T-effector cells[108]. The role of A_{2A} receptors on T_{Reg} cells was inferred by results showing that co-transfer of $CD4^+CD25^+$ T_{Reg} cells obtained from A_{2A} receptor wild-type mice was able to prevent disease induction in severe combined immunodeficiency mice that were transferred adoptively with disease-inducing $CD45RB^{high}$ $CD4^+$ T cells, a rodent model of IBD, whereas co-administration of $CD4^+CD25^+$ T_{Reg} cells isolated from A_{2A}-receptor-knockout mice in conjunction with disease-inducing $CD45RB^{high}$ $CD4^+$ T cells was inefficient in blocking the development of disease[48].

The observation that activation of A_{2B} receptors on intestinal epithelial cells can augment Il6 production by these cells resulting in increased neutrophil activation[109] combined with increased detection of A_{2B} receptors in epithelial cells isolated from mouse or human colitis[110] prompted recent studies assessing the effect of A_{2B} receptor blockade in IBD. Administering ATL-801, a selective A_{2B} antagonist, to mice suffering from colitis markedly decreased IL6 production and neutrophil infiltration, and reduced the extent of mucosal damage thereby ameliorating the course of disease[111]. This pro-inflammatory role of A_{2B} receptor

activation by endogenous adenosine was recently confirmed using A_{2B}-receptor-knockout mice[112], confirming that blockade of A_{2B} receptor represents a potentially advantageous treatment option that selectively targets the gut for patients afflicted with IBD. This proposition is predicated on the findings that A_{2B} receptor expression is highest in the gut when compared with other organs and that levels of endogenous adenosine that appear to perpetuate the disease process through A_{2B} receptors are elevated mostly locally in the gut[111].

Wound healing

In the skin, inflammation is part of a continuum in which injured tissue is repaired and replaced resulting in wound healing. Montesinos *et al.* first reported that topical application of adenosine A_{2A} receptor agonists increases the rate of wound healing in normal mice and in diabetic rats[113]. Increases in matrix and vessel formation were induced by adenosine receptor agonists and the improvement in the rate of wound healing was significantly greater than that induced by recombinant platelet-derived growth factor[113-116]. A_{2A} and A_{2B} receptor stimulation increases angiogenesis both directly and indirectly: adenosine receptors directly stimulate microvascular endothelial cell-proliferation as well as the autocrine production of vascular endothelial growth factor (VEGF), a central stimulus for endothelial proliferation and angiogenesis[117-119], and inhibit the production of thrombospondin 1, an anti-angiogenic matrix protein[120]. In addition, adenosine stimulates VEGF production by macrophages[116, 121,122]. Moreover, A_{2A} and A_{2B} receptor stimulation promotes matrix production by skin fibroblasts, an essential step in tissue repair[66,123-125]. In this context, it is noteworthy that A_{2A}-receptor-deficient mice do not form granulation tissue indicating that endogenous adenosine plays a central role in wound healing[114]. Sonedenoson (MRE0094), an A_{2A} agonist that regulates the inflammatory response and enhances tissue regeneration, is currently undergoing trials for the treatment of diabetic foot ulcers(TABLE 1).

Angiogenesis

The growth of new blood vessels from pre-existing vessels. Angiogenesis is a normal process in growth and development but it also a fundamental process required for the growth of tumours.

Box 2

Targeting adenosine receptors for treating inflammatory diseases

Asthma and chronic obstructive pulmonary disease (COPD)

- A_{2A} receptor agonists prevent inflammatory-cell infiltration into the lung and thus may be of therapeutic use. Potential problems may result from cardiovascular side effects with these agents.
- A_{2B} receptor antagonists prevent mast-cell degranulation and the overproduction of pro-inflammatory cytokines and extracellular matrix by smooth-muscle cells, bronchial epithelial cells and lung fibroblasts.

Ischaemia

- A_{2A} receptor agonists potently downregulate inflammatory-cell infiltration into tissues, production of deleterious free radicals and pro-inflammatory cytokines. Potential issues relate to their cardiovascular side effects.

Arthritis

- A_{2A} receptor agonists have a wide range of anti-inflammatory effects in the inflamed joint, but can exhibit cardiovascular side effects.

- A_3 receptor agonists decrease tumour necrosis factor-α production by monocytes and synoviocytes. Development of more selective and potent A_3 receptor agonists is needed.

Sepsis

- A_1 receptor agonists can prevent inflammation-mediated organ injury in animal models; however, their utility is limited by cardiovascular depressive effects.
- A_{2A} receptor antagonists are beneficial in sepsis by boosting eradication of bacteria. One potential problem is that sepsis is a heterogeneous group of diseases and inflammatory tissue injury rather then infection dominates the clinical picture in a smaller group of patients. A_{2A} receptor antagonists may exacerbate organ injury in these patients.

Inflammatory bowel disease

- A_{2A} receptor agonists attenuate inflammatory-cell sequestration in the gut and increase regulatory T-cell activity thereby ameliorating the course of disease. They may have cardiovascular side effects.
- A_{2B} receptor antagonists prevent intestinal epithelial-cell-mediated inflammatory events and thereby prevent mucosal inflammation.

Wound healing

- Topical administration of A_{2A} receptor agonists increases the rate of wound healing, in part, by stimulating angiogenesis in the skin. Thus, A_{2A} receptor agonists are good candidates for the treatment of diabetic foot ulcer.

Conclusions

The adenosine receptor system has evolved as both a rapid sensor of tissue injury and the major ‘first-aid’ machinery of tissues and organs. Adenosine receptor activation thus preserves tissue function and prevents further tissue injury following an acute injurious insult, such as reperfusion injury, actions in which the immune system has a paramount role. This primordial protective function of the adenosine receptor system following acute insults can, however, be overshadowed by its reduced ability to protect against chronic insults. In addition, in certain chronic disease states, such as asthma, the adenosine receptor system can even exacerbate tissue dysfunction.

Recent advances in understanding the role of the distinct adenosine receptors and the complex network of cellular players that determine the adenosine response to tissue injury have helped identify novel pharmacological targets to restore tissue function in various diseases(BOX 2). Despite growing enthusiasm regarding these targets, it is important to emphasize that because most of the data were obtained using animal models, caution should be exercised in translating results to the clinic. potential difficulties in translating results may stem from, in part, species differences and the fact that animal studies generally are done in young, relatively healthy animals over a short time-span. Of particular relevance is species differences in adenosine signalling in asthma studies. In rodents, primed mast-cell degranulation appears to be mediated primarily by A_3 receptors, whereas in humans A_{2B} receptors are more important.

Although adenosine receptor agonists have powerful immunomodulatory actions, the wide tissue distribution of adenosine receptors may limit their usefulness in the treatment of inflammatory diseases. Adenosine receptor antagonists, however, represent an ideal target for the therapy of certain immune-related disorders because their action is selectively targeted to

the site of injury, where endogenous adenosine is released. Similarly, interfering with the function of enzymes and transporters that are responsible for the accumulation of extracellular adenosine allows local targeting of adenosine receptors, providing an opportunity for interventions with limited side effects. It is clear that a better understanding of adenosine receptor function will be required before the enormous potential of adenosine-based therapies can be realized to ease human suffering.

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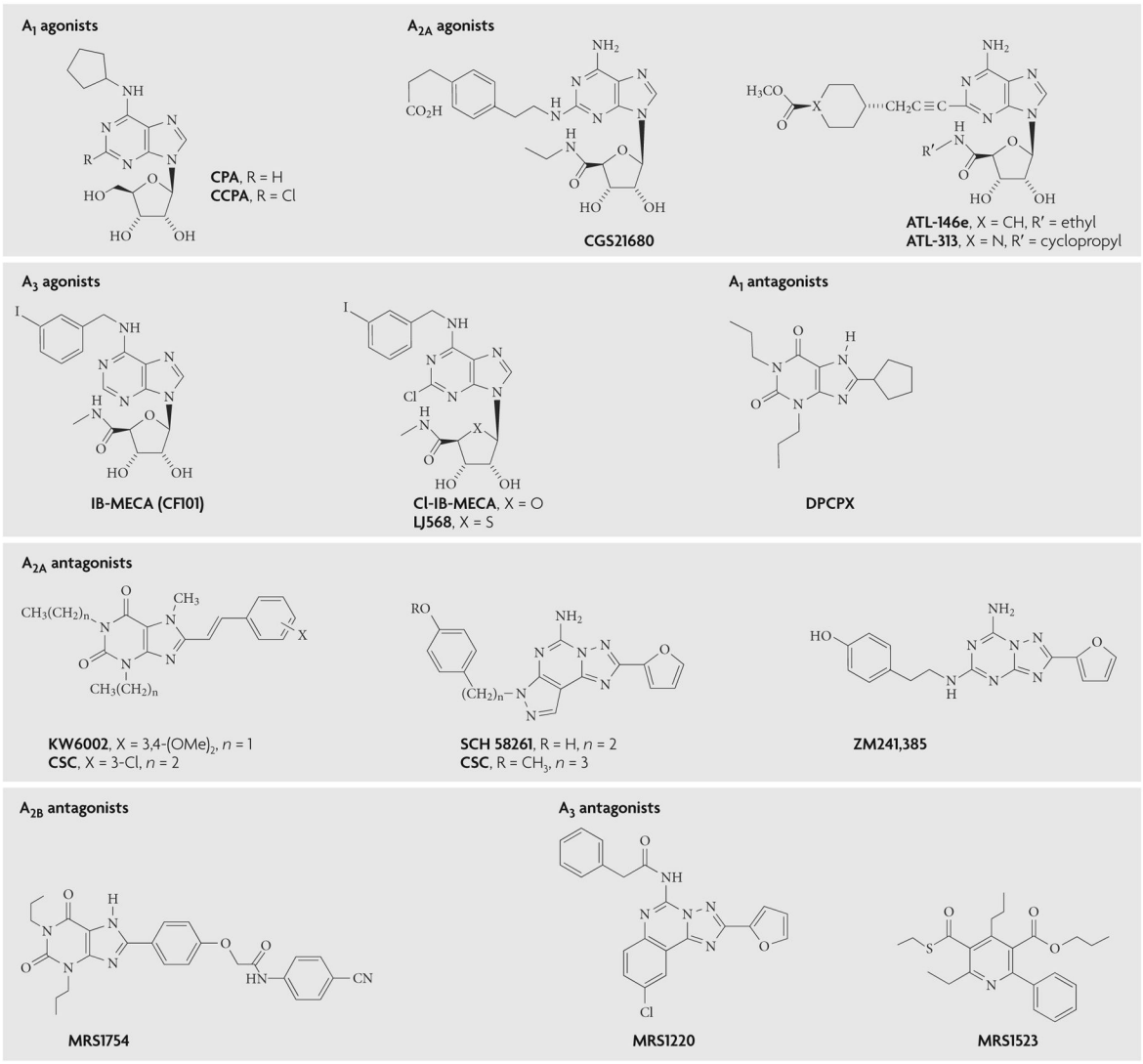

Figure 1. Representative adenosine receptor ligands

These ligands are the most widely used adenosine receptor agonists and antagonists in *in vitro* and *in vivo* studies assessing the function of adenosine receptors. IB-MECA (CF101) is currently undergoing testing in clinical trials for the treatment of rheumatoid arthritis.

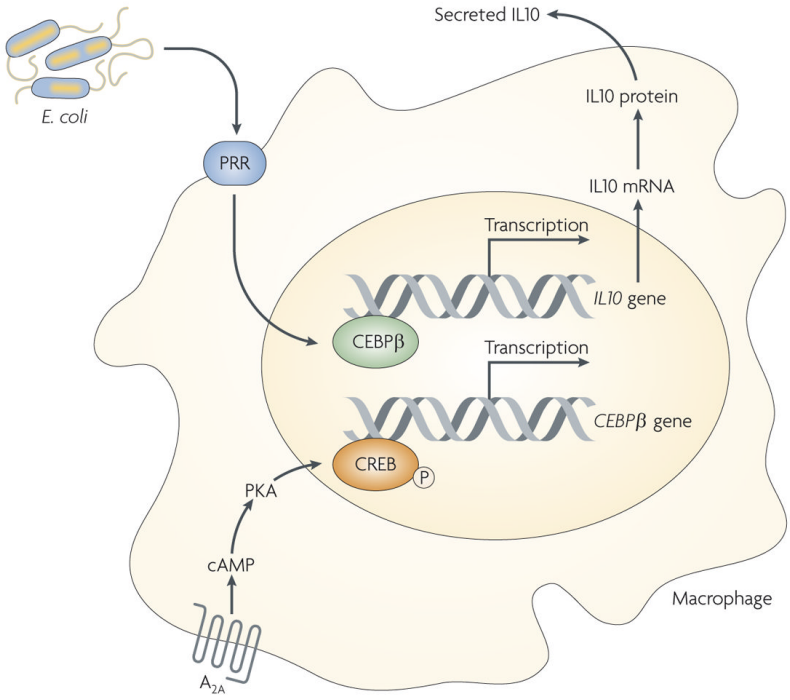

Figure 2. Pattern-recognition receptor-mediated and A_{2A} receptor-triggered pathways converge on CEBPβ to induce IL10 production by macrophages

A_{2A} receptor activation increases intracellular cyclic AMP (cAMP) levels resulting in increased protein kinase A (PKA) activation. PKA phosphorylates cAMP responsive element binding protein (CREB), which causes an increase in its transactivating potential leading to the transcription of the *CEBPβ* gene. CEBPβ protein binds to the *IL10* gene promoter, which triggers IL10 transcription and subsequently leads to the release of IL10. Components of *Escherichia coli* trigger activation of pattern-recognition receptors (PRRs) and bring about increased activation of CEBPβ.

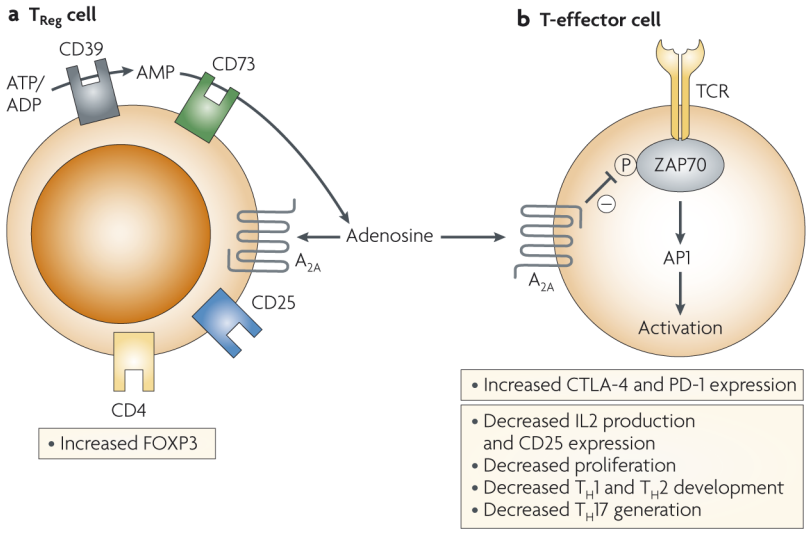

Figure 3. Mechanisms of T_{Reg} cell-mediated suppression of T-effector cells

Regulatory T (T_{Reg}) cells produce adenosine following sequential degradation of ATP/ADP via CD39 (ENTPD1; ectonucleoside triphosphate diphosphohydrolase 1) and CD73 (ecto-5′-nucleotidase) (**a**). Adenosine activates A_{2A} receptors on T-effector cells to inhibit T-cell receptor (TCR)-mediated signalling by preventing ZAP70 phosphorylation and activation of the transcription factor activator protein 1 (AP1) (**b**). This decreased TCR signalling leads to decreased interleukin 2 (IL2) production and CD25 expression resulting in decreased T effector cell proliferation. In addition, the development of both T helper 1 (T_H1) and T_H2 cells, as well as the generation of T_H17 lymphocytes is inhibited following A_{2A} receptor stimulation. A_{2A} receptor stimulation on T-effector cells increases expression of negative co-stimulatory molecules such as cytotoxic T-lymphocyte-associated antigen 4 (CTLA4) and programmed cell death 1 (PD1). A_{2A} receptor stimulation on T_{Reg} cells augments FOXP3 expression in these cells.

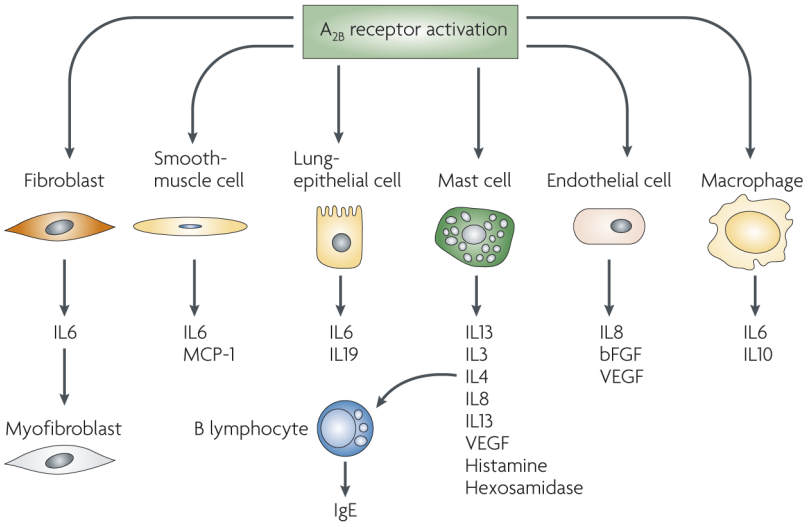

Figure 4. A_{2B} receptor activation has broad pro-inflammatory actions by stimulating the pro-inflammatory functions of a variety of cell types that mediate asthma

A_{2B} receptor activation increases interleukin 6 (IL6) production by pulmonary fibroblasts, which in turn leads to increased generation of myofibroblasts, which are capable of depositing extracellular matrix. A_{2B} receptor activation promotes the production of pro-inflammatory factors by mast cells and stimulates mast-cell degranulation. Increased IL4 production following mast cell A_{2B} receptor activation leads to increased immunoglobulin E (IgE) production by B cells. bFGF, basic fibroblast growth factor; MCP1, monocyte chemoattractant protein 1; VEGF, vascular endothelial growth factor.

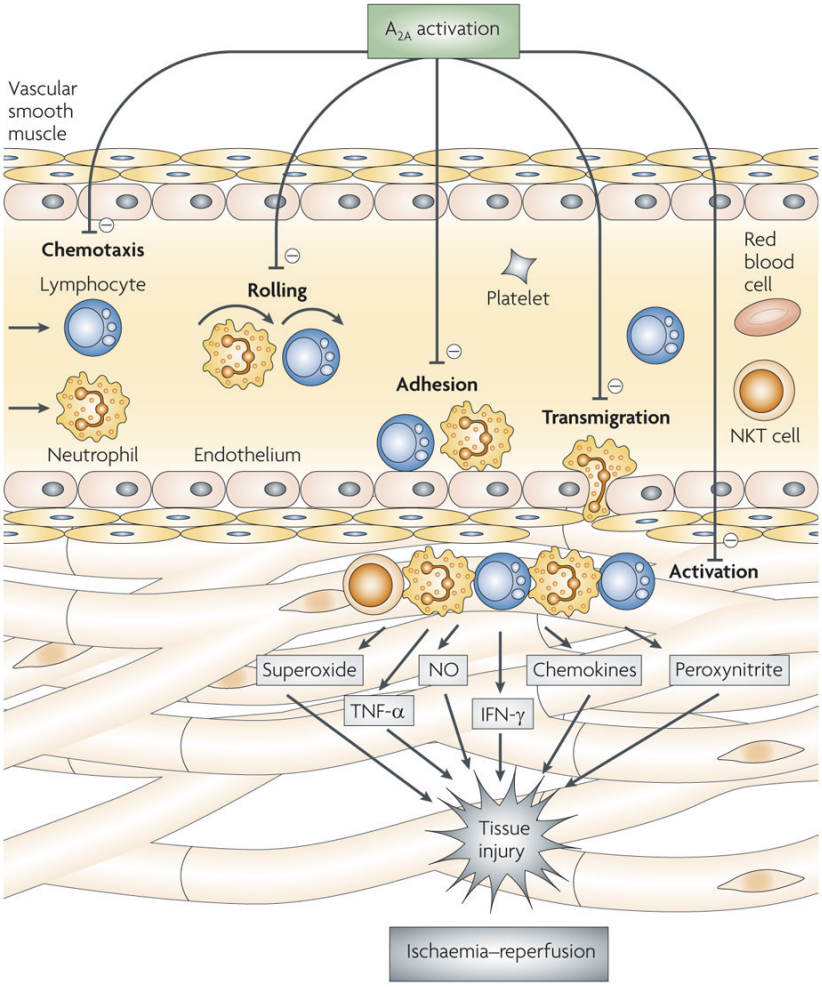

Figure 5. A_{2A} receptor activation protects organs from ischaemia–reperfusion injury by widely inactivating the ischaemia–reperfusion-induced inflammatory response

A_{2A} receptor activation reduces ischaemia–reperfusion-induced rolling, adhesion and transmigration of various inflammatory cells, including natural killer T (NKT) cells, lymphocytes and neutrophils. A_{2A} receptor stimulation also limits inflammatory cytokine and chemokine production, superoxide release and interferon-γ (IFN-γ) secretion by activated immune cells. NO, nitric oxide; TNF-α, tumour-necrosis factor-α.

Table 1

Adenosine receptor ligands in clinical studies for treating inflammatory diseases

Drug	Target receptor	Agonist or antagonist	Disease	Status	Company	References
CVT-6883	A_{2B}	Antagonist	COPD	Phase I	CV Therapeutics	Company web site‡
GW328267X	A_{2A}	Agonist	COPD	Phase II*	GlaxoSmithKline	72
UK-432097	A_{2A}	Agonist	COPD	Phase II	Pfizer	ClinicalTrials.gov Identifier NCT00430300‡
IB-MECA (CF-101)	A_3	Agonist	Rheumatoid arthritis	Phase II	CanFite BioPharmaceuticals	99
Sonedenoson (MRE-0094)	A_{2A}	Agonist	Diabetic foot ulcer	Phase II	King Pharmaceuticals	Company web site‡

*Discontinued.

‡See Further information for more details. COPD, chronic obstructive pulmonary disorder.